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(54) Title: METHOD OF REMOVING ENDOTOXIN CONTAMINANTS

(57) Abstract

A method for removing endotoxin contaminants from a biological material is provided. A biological material such as a protein preparation to be decontaminated is contacted with a detergent which solubilizes and removes endotoxin but which does not denature the active biologic component. The detergent can be a bile acid such as taurodeoxycholate or a bile acid/N-alkylsulfobetaine such as CHAPS. A chelating agent can be used with the detergent to remove divalent cations. The endotoxin content of protein preparations can be reduced to less than 0.1 EU/mg biological material by this method. The method is particularly applicable to the purification of immunoglobulins especially those that have a binding affinity for endotoxin such as anti-endotoxin antibody.

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METHOD OF REMOVING ENDOTOXIN CONTAMINANTS

Background of the Invention

Gram-negative endotoxins, also referred to as lipopolysaccharides (LPS), have potent biological effects in man that include pyrogenic and shock reactions. These substances are shed from the cell walls of viable and non-viable gram-negative bacteria. Because these bacteria are very hardy and grow in water with minimal nutrient requirements, endotoxin is a potential contaminant of physiological fluids and aqueous solutions, or the surfaces in contact with such substances.

Endotoxins are very stable molecules which survive extremes of temperature and pH. This fact makes decontamination or removal of endotoxins from solutions a problem in biological research as well as in the pharmaceutical industry. However, because of its potent biological activity, it is crucial to remove endotoxin from drugs and therapeutics. This problem is further heightened with the production of biologic therapeutics for the treatment of gram-negative bacteremia and sepsis.

The fusion of mouse myeloma cells to spleen cells, first demonstrated by Kohler and Milstein, allows the generation of continuous cell lines making homogeneous antibody (hereinafter referred to as monoclonal antibody, MAb). Subsequently, much effort has been directed toward the production of various hybrid cells (called hybridomas) and to uses

of MAb made by these hybrid cells. In this regard, MAb have been produced against the LPS cell wall moieties of gram-negative bacteria to be used in the treatment of gram-negative bacteremia and sepsis. However, the presence of contaminating endotoxins during the manufacture and processing of anti-LPS MAb results in the formation of an antibody-antigen complex which severely limits the clinical potential of the antibody.

LPS have self aggregating properties which result in large polydispersed molecular forms. LPS may be dispersed by detergents such as sodium dodecyl sulfate (SDS) and surfactants such as Triton X-100 or sodium deoxycholate. Such observations suggest that hydrophobic interactions between subunits of LPS are important determinants of particle size. Gram-negative LPS also have a high content of the cations, calcium (Ca^{2+}) and magnesium (Mg^{2+}). The removal of the cations reduces the size of LPS, and the readdition of divalent cations greatly enlarges particle size.

Methods for separating LPS from biological materials include the use of polymixin B which binds LPS. Generally, polymixin B is attached to a solid phase and the material to be purified is contacted with the solid phase to allow the LPS to be selectively adsorbed thereto. Another technique for removal of LPS is ion exchange chromatography. These techniques may not be effective for removal of sufficient endotoxin from biological material which

bind endotoxin either specifically or nonspecifically to provide preparations suitable for in vivo administration.

Summary of the Invention

This invention pertains to a method of removing endotoxin contaminants from preparations of biological material such as proteinaceous biological materials, particularly immunoglobulin preparations, under nondenaturing conditions so as to preserve the biological activity of the active component of the biological material. The method can be used to separate endotoxin from biological materials which bind endotoxin either specifically or nonspecifically. The invention also pertains to preparations of immunoglobulins which are substantially endotoxin-free and devoid of pyrogenic activity that can be prepared by the method of this invention.

The method of this invention employs a nondenaturing detergent such as a zwitterionic or anionic detergent to solubilize endotoxin contaminant in a preparation of biological material. Preferred detergents are bile salts such as taurodeoxycholate and deoxycholate and bile salt/N-alkylsulfobetaines such as 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfate (CHAPS) and 3-[(3-cholamidoproyl)-dimethylammonio-2-hydroxy]-1--propane sulfate (CHAPSO). The method comprises contacting the biological material to be

decontaminated with the nondenaturing detergent under conditions which allow the detergent to solubilize the endotoxin contaminants associated with the biological material and thereafter separating the biological material from the detergent. In a preferred embodiment, the detergent is used in combination with a chelating agent for removal of divalent cations from the biological material.

The method of this invention is suited for purification of proteinaceous biological materials particularly immunglobulins. Biological materials containing 3-5 Endotoxin Units (EU)/mg of biological material to over 20 EU/mg can be purified by the method. The nondenaturing conditions permit substantial retention of biological activity of the material. For instance, immunoglobulins purified by the method can retain 85% or more of their immunoreactivity.

The purified composition of a biological material, particularly proteinaceous material, is substantially endotoxin free and free of pyrogenic activity. The compositions have a total endotoxin content less than about 0.3 EU/mg of biological material preferably less than about 0.1 EU/mg and are substantially free of pyrogens as determined by the U.S.P. rabbit pyrogen test at a dosage of about 2 mg/kg rabbit weight preferably about 15 mg/kg rabbit weight. These compositions are useful for administration in vivo.

Detailed Description of the Invention

The method of the invention provides for the purification of biological material to yield preparations of such materials which are substantially free of bacterial endotoxin and pyrogenic activity. The purified materials are suitable for administration in vivo. The method is useful for the decontamination of proteinaceous material (e.g., proteins, peptides, and glycoproteins). The conditions employed are nondenaturing and do not significantly alter the biological activity of the active proteinaceous components. The method is particularly useful for the purification of immunoglobulins including monoclonal antibodies. Immunoglobulins purified by the method substantially retain their immunoreactivity.

The method can be used to remove endotoxin from preparations of biological material which have a specific or nonspecific affinity for endotoxin. For example, the method can be used to remove endotoxin from a preparation of anti-endotoxin antibodies. Anti-endotoxin antibodies are useful in therapy and prophylaxis of gram negative bacterial infection and in the in vivo imaging of gram negative bacterial abscesses. See e.g., International Patent Application PCT US84 00688; International Patent Application PCT US84 01643. Because the antibodies specifically bind endotoxin (preferably with high affinity), the removal of endotoxin from preparations of the antibodies, without destruction of

immunoreactivity, is difficult. The method of this invention provides for the purification of anti-endotoxin antibody without significant loss of biological activity.

According to the method of this invention, the biological material to be decontaminated of endotoxin is contacted with a detergent capable of solubilizing endotoxin contaminants without denaturing the biologically active component in an amount and under conditions sufficient to remove the endotoxin in the preparation. The biological material is then separated from the detergent with the endotoxin being removed with the detergent.

The detergent employed is a nondenaturing detergent capable of solubilizing endotoxin contaminants. Detergents from at least two classes are useful in the method: bile salts such as taurodeoxycholate and deoxycholate and detergents which are combinations of bile salts and N-alkylsulfobetaines such as the CHAPS and CHAPSO detergents. The CHAPS detergent is particularly preferred; it is a zitterionic agent which can disaggregate proteinaceous material and solubilize endotoxin contaminants associated with the protein without denaturing the protein or significantly disrupting its charge properties so as to preserve biological activity. Anionic or cationic detergents which solubilize endotoxin contaminants but do not denature or significantly alter the charge properties

of the material to be purified can also be used in the method of this invention such as the anionic bile salt, taurodeoxycholate mentioned above. Other detergents or surfactants useful in the method can be readily ascertained empirically.

In typical procedures, the detergent is added to an aqueous buffer to provide a wash solution which is contacted with the biological material to be purified. Suitable buffers include Tris-HCl, phosphate buffered saline, or any of the conventional physiologically acceptable buffers which have buffering action at pH's within the range tolerated by the biological material to be purified. The amount of detergent added in solution is that amount capable of solubilizing and removing essentially all of the endotoxin contaminants from the biological material to be purified. For most applications, the amount of detergent ranges from 0.1-1% (w/v) of the buffer solution, preferably from 0.1-0.5% (w/v). Generally, this is an amount sufficient to reduce endotoxin content to less than 0.3 EU/mg of material preferably less than 0.1 EU/mg. The quantity of detergent solution needed to remove endotoxin can be readily determined empirically for any amount of biological material to be purified.

In a preferred embodiment a chelating agent is employed in conjunction with the detergent for removal of divalent cations (e.g. Ca^{++}, Mg^{++}) from the biological material. Preferred chelating agents are (ethylenedinitrilo)-tetraacetic acid (EDTA) and ethylene glycol-bis-(β -aminoethyl ether)-N,N,N',N'-

tetraacetic acid (EGTA). The chelating agent is employed in an amount sufficient to chelate and to remove essentially all of the divalent cations in the preparation of biological material to be purified. In general an excess of chelating agent is employed to accomplish this. Most often, EDTA is employed at a concentration of about 10 mM.

The washing step can be performed as follows. In one embodiment, the preparation of biological material is combined with a solution of detergent and chelating agent. The combination is mixed and incubated. The biological material can be separated from the detergent solution by precipitation or by chromatographic techniques (e.g. ionic exchange chromatography, gel filtration chromatography).

Alternatively, the biological material can be immobilized onto a solid phase before contact with the solution of detergent and chelating agent to facilitate the washing and separation steps of the method. For example, an immunoglobulin preparation can be bound to the solid phase and then washed with the solution containing the detergent and the chelating agent. Suitable solid phases for immobilization of immunoglobulin include ionic exchange resins such as Mono S or Sepharose Fast Flow from Pharmacia Inc. Piscataway, NJ.

After separation from the wash solution the biological material may be further purified by chromatography, filtration and/or dialysis.

The method of this invention can be used to remove endotoxin from biological materials having levels of endotoxin contamination of from 2-5 EU/mg to over 20 EU/mg biological material. The method provides a purified composition of a biological material which is useful for in vivo administration. The composition has a total endotoxin content less than about 0.3 EU/mg of material and is substantially free of pyrogens as determined by the U.S.P rabbit pyrogen test at a dosage of about 15 mg/kg rabbit weight. Preferably, the total endotoxin content is less than about 0.1 EU/mg of material.

The method of this invention can be used to purify immunoglobulins such as preparations of anti-endotoxin antibody which have a binding affinity for endotoxin. Immunoreactivity of at least 85% can be retained. For example, the method can be used to remove endotoxin from human monoclonal anti-endotoxin antibody to yield an antibody preparation having less than 0.3 EU/mg, preferably less than 0.1 EU/mg, with retention of at least 85%, of its immunoreactivity.

This invention is illustrated further by the following examples.

EXAMPLES

Anti-Endotoxin MAb preparation

The mouse-human heteromyeloma cell line SHMD33 fused with Epstein Barr Virus transformed human

spleen lymphoid cells gave rise to the HA-1A cell line which expressed the IgM monoclonal antibody (MAb), anti-J5 C9, hereinafter referred to as HA-1A. HA-1A has been shown to bind to the Lipid A portion of a large class of lipopolysaccharides, which are cell wall component of several genera of gram-negative bacteria.

Large scale production of the HA-1A MAb was undertaken by Endotronics, Inc., Alburquerque, NM, to provide tissue culture supernatant of the HA-1A cell line. This tissue culture supernatant was concentrated 10-fold by a Pellicon ultrafiltration system employing a 100,000 molecular weight cut-off membrane. Polyethylene glycol 6000 (PEG 6000) was added to the concentrated supernatant to a final concentration of 4% w/v. This was allowed to incubate at 4°C overnight at which time the precipitate was collected by centrifugation and dialyzed into 50mM (2[N-Morpholino] ethanesulfonic acid) (MES) buffer containing 200 mM NaCl at a pH of 6.2. The solubilized material was then diluted 1:1 with 50 mM MES, pH 6.2, containing 0mM NaCl and applied to a strong cation exchange resin, S-Sepharose Fast Flow, obtained from Pharmacia, Inc, Piscataway, NJ. Contaminants were allowed to pass through the column by elution with 50 mM MES, pH 6.2. Elution of the bound HA-1A MAb from the resin was accomplished by employing a gradient of 100-300 mM NaCl. The HA-1A was shown to elute at approximately 120 mM NaCl. The HA-1A containing

fractions were then pooled and immediately dialyzed into 50 mM tris, 0.3 M NaCl at a pH of 8.0. This partially purified MAb was then passed over a Q-Sepharose Fast Flow anion exchange column in 50 mM tris, 0.3 M NaCl at pH 8.0. These conditions allow DNA and free endotoxin to bind to the resin while the HA-1A MAb will not be retained. These partially purified preparations of the HA-1A MAb designated LOT 02516 and LOT 03036 were found to have an endotoxin contamination level of 23 and 17 endotoxin units/mg of Ab (EU/mg), respectively, as determined by the chromogenic limulus amebocyte lysate (LAL) assay for use in the quantification of endotoxin, were used in the following examples of endotoxin removal.

The extraction method of Bligh-Dyer was employed to isolate the contaminant(s) which gave rise to the positive LAL value. Thin-layer chromatography on silica gel of the organic phase of the extraction procedure was performed using a mixture of chloroform:methanol:water (65:35:4) as the mobile phase and iodine as the developing reagent. The contaminant had an R_f value which most closely resembled dimyristylphosphatidylcholine or a single component of LPS from E. coli 055:B5. A complete list of the compounds included in the experiment and their corresponding R_f values is found in Table I.

TABLE I

Thin Layer Chromatography on silica gel
Developed in $CHCl_3$:MeOH:H_2O

Sample	R_f (x100)
HA-1A (LOT 02516)	31
E. coli 055:B5 LPS	0, 11, 20, 33
phosphatidyl inositol	17
Dimyristylphosphatidyl serine	15
Dimyristylphosphatidyl choline	34
Dimyristylphosphatidyl glycerol	48
Dimyristylphosphatidyl ethanolamine	62
Dimyristylphosphatidic acid	72

Limulus Amebocyte Lysate (LAL) Assay

The LAL activity assay was measured using a commercially available kit QCLAL Whittaker/MA Bioproducts, Walkersville, MD. The assay involves incubation of the test sample containing endotoxin with a lysate prepared from the circulating amebocytes of the horseshoe crab Limulus polyphemus. The endotoxin catalyzes the activation of a proenzyme in the lysate. The activated enzyme catalyzes the splitting of para-nitroaniline (pNA) from the colorless substrate Ac-Ile-Glu-Gly-Arg-pNA. The pNA released is measured photometrically at 405 nm after the reaction is quenched by addition of acetic acid. The correlation between the absorbance and the endotoxin concentration is linear in the 0.1-1.0 EU/ml range. The concentration of endotoxin in a sample is calculated from the absorbance values of solutions containing known amounts of endotoxin standards.

Rabbit Pyrogen Test

Rabbit pyrogen test was performed under GLP by an outside testing laboratory. Each of three rabbits was weighed, and its weight recorded to the nearest 0.1 grams. The volume to be inocculated for each rabbit was calculated based on the test doses in ml/kg, not to exceed 10ml/kg. The HA-1A preparation was prewarmed to 37°C. The rectal temperature

of each rabbit was taken with a clinical thermometer. Only rabbits whose control temperatures did not deviate more than 1°C from each other and did not exceed 39.8°C were used. Within 30 minutes of measuring the control temperature each rabbit was injected I.V. in the ear vein with the calculated volume. The rectal temperatures were measured at one, two, and three hours post injection and recorded to the nearest 0.1°C. The HA-1A was considered to have met the requirements for absence of pyrogens if no rabbit showed a temperature rise of 0.6°C or greater above its control temperature at any time period and the sum of the maximum temperature rises of the three rabbits did not exceed 1.4°C.

EXAMPLE I. Depyrogenation of HA-1A by incubation with 3-[(3-cholamidopropyl-dimethyl ammonio]-1-propane sulfonate/(ethylenedinitrilo) tetraacetic acid (CHAPS/EDTA) and isolation by PEG 6000 precipitation.

A 0.5 ml aliquot of the contaminated HA-1A MAb (LOT 02516, 23 EU/mg, 0.57 mg/ml) in 50 mM tris, 0.3 M NaCl, pH 8.0 was combined with 0.5 ml of a 20 mM CHAPS-20 mM EDTA solution in 0.9% NaCl, pH 7.0. As a control, an equal volume of the contaminated HA-1A MAb (LOT 02516, 23 EU/mg, 0.57 mg/ml) in 50 mM tris, 0.3 M NaCl, pH 8.0 was combined with 0.5 ml of 0.9% NaCl, pH 7.0.

Incubation at 37°C was allowed to proceed for 30 minutes at which time PEG 6000 was added to a final concentration of 4% w/v. These mixtures were kept at 4°C for 90 minutes at which time the solutions were centrifuged and the PEG precipitates collected. The PEG precipitate was then resuspended in 1 ml of 0.9% NaCl and washed (2x). Final recovery of the CHAPS/EDTA untreated sample and the purified HA-1A were 78 and 83%, respectively, as judged by absorbance at 280 nm. The LAL level of the purified HA-1A had been reduced by 94% to 1.3 EU/mg of Ab while the LAL level of the untreated sample remained at 23.3 EU/mg. In addition, the immunoreactivity of the treated HA-1A was shown to increase approximately 50% as evidenced by a particle fluorescent concentration immunoactivity assay (PFCIA). Briefly, the assay employs incubation of Lipid A or LPS coated latex particle beads with several dilutions of HA-1A antibody solution. Following a washing step bound HA-1A antibody is incubated with FITC-labeled goat anti-human IgM F_c. Quantitation of the bound fluorescent probe is a measure of the immunoreactivity of the antibody.

EXAMPLE II. Depyrogenation of HA-1A by washing ion-exchange resin immobilized MAb with a CHAPS/EDTA mixture.

HA-1A (LOT 02516, 1.0 mg/ml, 5 mgs, 23 EU/mg) was diafiltered in an Amicon flow cell until buffer exchange into 50 mM MES, pH 6.2, 200 mM NaCl was

achieved (10 vols). Dilution with an equal volume of 50 mM MES, pH 6.2, 0 mM NaCl was performed immediately preceeding application of the sample to a HR 5/5 Mono S cation exchange column attached to an FPLC system, both obtained from Pharmacia, Inc. The 10 mM CHAPS/10 mM EDTA mixture dissolved in 50 mM MES, pH 6.2, 100 mM NaCl (10 mls, 5 column volumes) was applied to the column via a de-pyrogenated 10 ml superloop. Following elution of this detergent/chelate mixture the column was equilibrated with 50 mM MES, pH 6.2, 100 mM NaCl. Residual CHAPS was not detected by thin layer chromatography at a level of 0.5 umole. The HA-1A MAb was then eluted from the column with 50 mM MES, pH 6.2, 200 mM NaCl. Endotoxin activity in the eluted antibody preparation as measured by the LAL assay, was less than 0.1 EU/mg. This represented greater than 99% removal of the endotoxin from the anti-lipid A antibody. The endotoxin was shown to elute during the column washes of the CHAPS/EDTA mixture. Again, as in the PEG 6000 precipitation of Example I, immunoreactivity appeared to be increased almost 2-fold as measured by the PCFIA assay. High performance liquid chromatography (HPLC) employing a silica gel matrix for gel permeation chromatography showed both endotoxin contaminated and purified antibody to exhibit similar profiles and molecular weight. In addition, isoelectric focussing (IEF) of the contaminated and purified Ab showed identical isoelectric points (pI = 6.1).

EXAMPLE III. Depyrogenation of HA-1A MAb by washing ion-exchange resin immobilized MAb with a tauro-deoxycholate/EDTA mixture.

HA-1A (lot 70048) was precipitated from a 5X concentrated solution of tissue culture supernatant by incubation with 4% polyethylene glycol 6000 (PEG 6000) overnight at 4°C. The HA-1A precipitate was redissolved in 50 mM MES, pH 6.2, 200 mM NaCl and a portion (20 mg) after dilution with an equal volume of 50 mM MES, pH 6.2, 0 mM NaCl was partially purified by application to an HR 5/5 S-Sepharose Fast Flow ion-exchange column obtained from Pharmacia, Inc. Approximately 50% of the total protein bound to the column and was eluted with a salt gradient of 100-300 mM NaCl dissolved in 50 mM MES, pH 6.2. The HA-1A peak was pooled and brought to 0.2 M NaCl by addition of 500 mM NaCl, 50 uM MES, pH 6.2. This eluate had an endotoxin level of 160 EU/mg. Subsequent dilution of this fraction to 100 mM NaCl, 50 mM MES, pH 6.2 and application to the HR 5/5 S-Sepharose Fast Flow column resulted in total binding. The column was washed with 5 column volumes of 100 mM NaCl, 50 mM MES, pH 6.2. Elution of the salt washed HA-1A gave rise to an endotoxin value of 6.1 EU/mg. This value is significantly lower than the 160 EU/mg value of the PEG 6000 precipitated material but it is not below acceptable limits for pyrogen levels for injectables. The 6.1 EU/mg HA-1A material was bound to a S-Sepharose Fast Flow column and washed with 5 column volumes of 10

mM taurodeoxycholate/10 mM EDTA in 50 mM MES, pH 6.2 buffer. Elution with 200 mM NaCl, 50 mM MES, pH 6.2 buffer gave rise to HA-1A material that had an endotoxin level the detectable limit of 0.1 EU/ml of the quantitative chromogenic LAL assay. In order to show that the tauro-deoxycholate/EDTA mixture was specifically removing LPS from the HA-1A MAb, the LOT 7D048 HA-1A 5X concentrated tissue culture supernatant was spiked with 20 EU/mg of authentic 0111:B4 LPS. Endotoxin levels of the 0111:B4 spiked HA-1A preparations following exactly the same protocol as above were: initial S-Sepharose partial purification, 251 EU/mg; S-Sepharose binding and mock wash with 100 mM NaCl, 50 mM MES, pH 6.2, 26.3 EU/mg: and following S-Sepharose binding and washing with 10 mM taurodeoxycholate/10 mM EDTA in 50 mM MES, pH 6.2, below the 0.1 EU/ul limit of detection of the LAL assay.

EXAMPLE IV. Removal of endotoxin from HA-1A comparing large scale, beta-hydroxymyristic acid assay for LPS, and rabbit pyrogen testing.

HA-1A (LOT 02516, 23 EU/mg, 1 mg/ml, 30 mg) was bound to a HR 5/5 Mono S column essentially according to example II except that six times the amount of antibody was bound to the cation-exchange resin and therefore, 40 mls of the 10 mM CHAPS/10 mM EDTA mixture was used to wash the bound antibody. As before, 200 mM NaCl dissolved in 50 mM MES buffer, pH 6.2 was used to elute the MAb. The

purified Ab was buffer exchanged into 10 mM sodium phosphate, 300 mM NaCl, pH 7.4 via an Amicon flow cell equipped with a 50,000 molecular weight cut-off membrane. Endotoxin contamination in the purified HA-1A antibody preparation was measured to be 0.17 EU/mg as determined by the LAL assay. The result indicates a 99% removal of the endotoxin from the HA-1A sample. This result was corroborated by a separate assay which measures beta-hydroxy myristic acid a specific component of lipopolysaccharides. This assay was performed essentially according to Maitra S. et al., Proc. Natl. Acad. Sci. USA 75:3993-3997 (1978). Briefly, the sample is hydrolyzed with hydrochloric acid, free fatty acids are extracted with diethyl ether, converted into their methyl esters and then into their trimethyl-silyl derivatives. The sample is then quantitated and analyzed by gas chromatography-mass spectroscopy. This procedure showed there to be 96% removal of the contaminating lipopolysaccharide, as measured by quantitation of beta-hydroxy myristic acid. The CHAPS/EDTA treated HA-1A MAb had only 13.4 ng of LPS per mg of antibody as compared to the original material which contained 296 ng LPS/mg Ab. In addition, the purified CHAPS/EDTA treated HA-1A MAb was tested for and passed the rabbit pyrogen test at a human equivalent dosage of 100 mg/kg. Previously, the untreated HA-1A (LOT02516) which measured 23 EU/mg had passed the rabbit pyrogen test

at a human equivalent dosage of 25 mg/kg but had failed at the 100 mg/kg level.

EXAMPLE V. Comparison of Components of CHAPS/EDTA mixture to remove endotoxin from HA-1A.

In three separate experiments, 10 mg of HA-1A MAb (23 EU/mg, 1 mg/ml, LOT 02516) was applied to a HR 5/5 Mono S ion-exchange column according to examples II and IV in order to compare the efficacy of 10 mM CHAPS in 50 mM MES, pH 6.2, of 10 mM EDTA in 50 mM MES, pH 6.2 and of the 10 mM CHAPS/10 mM EDTA mixture in 50 mM MES, PH 6.2 to purify the HA-1A MAb from endotoxin. Following these washes the HA-1A was eluted from the Mono S column with 200 mM NaCl dissolved in 50 mM MES, pH 6.2. The endotoxin levels of the various treatments showed complete removal of LAL activity by both treatment with 10 mM CHAPS in 50 mM MES, pH 6.2 and by the 10 mM CHAPS, 10 mM EDTA in 50 mM MES, pH 6.2 mixture. Approximately 98% of the LAL positive contaminants were removed by the 10 mM EDTA in 50 mM MES, pH 6.2 treatment.

EXAMPLE VI. Recovery Experiment.

Purified HA-1A MAb (LOT 02516, 0.17 EU/mg, 20 mg) was combined with 40 ng of LPS from E. coli 0111:B4 (final endotoxin value of 20 EU/mg of HA1A) and allowed to incubate at 37° for 4 hours. One-half (10 mg HA-1A) of this mixture was applied to a HR 5/5 Mono S ion-exchange column as in EXAMPLE

II and eluted with 200 mM NaCl, 50 mM MES, pH 6.2 without treatment with the 10 mM CHAPS/10 mM EDTA mixture. The other half endotoxin spiked HA-1A samples (5 mg) was applied to the Mono S column, as above, but was then treated with the 10 mM CHAPS/10 mM EDTA mixture and then eluted with 200 mM NaCl, 50 mM MES pH 6.2. The LAL activity of the untreated HA-1A eluate was 21.1 EU/mg, essentially unchanged from the 20.0 EU/mg input, while the CHAPS/EDTA treated eluate exhibited an activity of just 1.2 EU/mg.

EXAMPLE VIII. 17 EU/mg LOT of HA-1A purified of endotoxin by CHAPS/EDTA treatment.

HA-1A MAb (LOT 03036, 1 mg/ml, 17 EU/mg, 25 mg) was applied to a HR 5/5 Mono S column. In a mock treatment experiment, the column was equilibrated in 100 mM NaCl in 50 mM MES, pH 6.2 and then eluted with 200 mM NaCl in 50 mM MES, pH 6.2. The LAL activity of the eluted antibody was 92% of the original 17 EU/mg level. A similar application of the HA-1A antibody bound to the Mono S column following 10 mM CHAPS/10 mM EDTA in 50 mM MES, pH 6.2 treatment and elution with 200 mM NaCl in 50 mM MES, pH 6.2 gave an LAL value of 0.14 EU/mg. This represented a 99% removal of the endotoxin from the contaminated material. These results are similar to those of LOT 02516 (Example II) which showed a reduction of endotoxin to a level had a LAL value of 0.17 EU/mg of HA-1A MAb.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method of removing endotoxin contaminants from a preparation of biological material, comprising the steps of:
   a. contacting the biological material with a nondenaturing detergent in an amount sufficient to solubilize the endotoxin contaminants; and
   b. separating the biological material and the detergent.

2. A method of Claim 1, wherein the biological material is a proteinaceous material.

3. A method of Claim 1, wherein the proteinaceous material is an immunoglobulin.

4. A method of Claim 3, wherein the immunoglobulin is an anti-endotoxin antibody.

5. A method of Claim 4, wherein the anti-endotoxin antibody is HA-1A antibody.

6. A method of Claim 1, wherein the detergent is a bile acid or a bile salts/N-alkysulfobetaine.

7. A method of Claim 6, wherein the detergent is 3-[(3-cholamidopropyl)-dimethyl-

ammonio]-1-propanesulfate or 3-[(3-chloamidopropyl)--dimethylammonio]-2-hydroxy-1-propanesulfate.

8. A method of Claim 6, wherein the detergent is taurodeoxycholate or deoxycholate.

9. A method of Claim 1, wherein the biological material is immobilized on a solid phase.

10. A method of Claim 1, wherein the detergent is contained in an aqueous buffer.

11. A method of Claim 10, wherein the detergent is present in the aqueous buffer in an amount ranging from 0.1 - 1.0 percent (w/v).

12. A method of Claim 1, wherein the biological material is additionally contacted with a chelating agent for divalent cations.

13. A method of removing endotoxin contaminants from a preparation of proteinaceous material, comprising the steps of:

a. contacting the preparation of proteinaceous material with a solution comprising an aqueous buffer containing a nondenaturing detergent and a chelating agent for divalent cations;

b. separating the proteinaceous material and the buffer.

14. A method of Claim 11, wherein the proteinaceous material is an immunoglobulin.

15. A method of Claim 11, wherein the immunoglobulin is a monoclonal antibody.

16. A method of Claim 12, wherein the monoclonal antibody is an anti-endotoxin antibody.

17. A method of Claim 13, wherein the detergent is bile salt or bile salt/N-alkyl sulfobetaine.

18. A method of Claim 17, wherein the detergent is 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfate or 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfate.

19. A method of Claim 17, wherein the detergent is taurodeoxycholate or deoxycholate.

20. A method of Claim 13, wherein the amount of detergent is from 0.1 - 1.0% (w/v) of the aqueous buffer.

21. A method of Claim 13, wherein the proteinaceous material is immobilized on a solid phase.

22. A method of Claim 13, wherein the chelating agent is (ethylenedinitrilo)tetraacetic acid.

23. A method of removing endotoxin contaminants from a preparation of monoclonal anti-endotoxin antibody, comprising the steps of:

a. contacting a preparation of monoclonal antibody with an a wash solution comprising an aqueous buffer containing a chelating agent for divalent cations and a detergent selected from the group consisting of 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfate and taurodeoxycholate;

b. separating the monoclonal anti-endotoxin antibody and the wash solution.

24. A method of Claim 23, wherein the detergent comprises from 0.1 to about 0.5 percent (w/v) of the buffer solution.

25. A method of Claim 23, wherein the chelating agent is (ethylenedinitrilo)tetraacetic acid.

26. A method of Claim 23, wherein the anti-endotoxin antibody is immobilized on a cationic resin.

27. A method of removing endotoxin contaminants from a preparation of anti-endotoxin monoclonal antibody, comprising the steps of:

a. providing a preparation of anti-endotoxin monoclonal antibody to be decontaminated;

b. immobilizing the antibody on a solid phase;

c. washing the immobilized antibody with a solution comprising:

i) an aqueous buffer,

ii) a nondenaturing detergent selected from the group consisting of 3-[(cholamidopropyl)-dimethylammonio]-1-propanesulfate and taurodeoxy-cholate; and

iii) a chelating agent for divalent cations; and

d. thereafter removing the antibody from solid phase.

28. A purified preparation of an immunoglobuin having a specific or nonspecific binding affinity for endotoxin prepared by the method comprising:

a. providing a preparation of an immuno-globulin having a specific or nonspecific binding affinity for endotoxin, the preparation containing a level of endo-toxin contamination above about 2-5 EU/mg immunoglobulin;

b. contacting the immunoglobulin preparation with nondenatured detergent in an amount sufficient to solubilize endotoxin; and

c. separating the immunoglobulin and the detergent to provide an purified immunoglobulin preparation having an endotoxin content of less than about 0.3 EU/mg immunoglobulin wherein the immunoglobulin retains about 85% or more of its immunoreactivity.

29. An immunoglobulin of Claim 28 which is an anti-endotoxin antibody.

30. A method of Claim 29, wherein the anti-endotoxin antibody is HA-1A antibody.

31. A method of Claim 28, wherein the detergent is a bile acid or a bile salts/N-alkysulfobetaine.

32. A method of Claim 28, wherein the detergent is 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfate or 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfate.

33. A method of Claim 28, wherein the biological material is additionally contacted with a chelating agent for divalent cations.

34. A preparation of human anti-endotoxin antibody having an endotoxin level less than about 0.3 EU/mg antibody.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03773

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 P 21/00; A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K; C 12 P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Chemical Abstracts, vol. 75, no. 23, 6th December 1971 (Columbus, Ohio, US) C. Schnaitman: "Effect of ethylenediaminetetraacetic acid, Triton X-100, and lysozyme on the morphology and chemical composition of isolated cell walls of Escherichia coli" see page 90, abstract no. 137961g & J. Bacterial. 1971, 108(1), 553-63 --	1-3,9-15, 20,21
Y	Chemical Abstracts, vol. 93, no. 24, 15th December 1980 (Columbus, Ohio, US) see page 316, abstract no. 225618c & JP, A, 8062902 (TORAY INDUSTRIES, INC.) 12 May 1980 --	1-3,9-15, 20,21
A	Chemical Abstracts, vol. 107, no. 20, 16th November 1987 (Columbus, Ohio, US) R.S. Stinson et al.: "Removal of lipopolysaccharide from acellular Bordetella pertussis vaccine by	

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21st February 1989	05.04.89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	detergent treatment", see page 481, abstract no. 183396d & J. Biol. Stand. 1986, 14(4), 261-71 --	
A	Chemical Abstracts, vol. 81, no. 23, 9th December 1974 (Columbus, Ohio, US) J.R. Chipley: "Release of lipopolysaccharide, phospholipids, and enzymes from Salmonella enteritidis by ethylene-diaminetetraacetic acid", see page 222, abstract no. 148207r & Microbios 1974, 10(38-39), 139-50 --	
A	GB, A, 2053233 (INSTITUT PASTEUR) 4 February 1981	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8803773 SA 25249

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 23/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 2053233	04-02-81	FR-A,B 2460139	23-01-81
		DE-A- 3024282	08-01-81
		US-A- 4402939	06-09-83

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82